Research Article

Identification of Key Genes and Candidated Pathways in Human Autosomal Dominant Polycystic Kidney Disease by Bioinformatics Analysis

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Keywords

Autosomal dominant polycystic kidney disease · Bioinformatics analysis · Key genes · Metabolic pathways

Abstract

Background/Aims: Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic form of kidney disease. High-throughput microarray analysis has been applied for elucidating key genes and pathways associated with ADPKD. Most genetic profiling data from ADPKD patients have been uploaded to public databases but not thoroughly analyzed. This study integrated 2 human microarray profile datasets to elucidate the potential pathways and protein-protein interactions (PPIs) involved in ADPKD via bioinformatics analysis in order to identify possible therapeutic targets. **Methods:** The kidney tissue microarray data of ADPKD patients and normal individuals were searched and obtained from NCBI Gene Expression Omnibus. Differentially expressed genes (DEGs) were identified, and enriched pathways and central node genes were elucidated using related websites and software according to bioinformatics analysis protocols. Seven DEGs were validated between polycystic kidney disease and control kidney samples by quantitative real-time polymerase chain reaction. **Results:** Two original human microarray datasets, GSE7869 and GSE35831, were integrated and thoroughly analyzed. In total, 6,422 and 1,152 DEGs were extracted from GSE7869 and GSE35831, respectively, and of these, 561 DEGs were consistent between the databases (291

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upregulated genes and 270 downregulated genes). From 421 nodes, 34 central node genes were obtained from a PPI network complex of DEGs. Two significant modules were selected from the PPI network complex by using Cytotype MCODE. Most of the identified genes are involved in protein binding, extracellular region or space, platelet degranulation, mitochondrion, and metabolic pathways. **Conclusions:** The DEGs and related enriched pathways in ADPKD identified through this integrated bioinformatics analysis provide insights into the molecular mechanisms of ADPKD and potential therapeutic strategies. Specifically, abnormal decorin expression in different stages of ADPKD may represent a new therapeutic target in ADPKD, and regulation of metabolism and mitochondrial function in ADPKD may become a focus of future research.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic disorder of the kidney and is characterized by renal cyst formation [1]. ADPKD affects 12.5 million people worldwide among all ethnic groups and is the fourth leading cause of end-stage renal disease (ESRD) requiring renal replacement therapy worldwide [2]. In China alone, there are an estimated 1.5 million patients with ADPKD [3], and 4.7% of ESRD cases in Shanghai are attributed to ADPKD [4].

ADPKD is commonly caused by mutations in 2 genes, the polycystic kidney disease 1 (*PKD1*) [5] and 2 (*PKD2*) [6] genes. Functional loss of polycystin-1 or polycystin-2, the gene products of *Pkd1* and *Pkd2*, respectively, leads to cyst initiation and expansion, through abnormalities in a variety of intracellular signaling pathways [7, 8]. During the past 40 years, great advances have been made in understanding how ADPKD develops and how it can be diagnosed [9] and treated [10–13]. Notably, ADPKD is a common inherited disorder [14], and genetic mutations are central to exploring the underlying mechanisms of the disease.

High-throughput microarray analysis is a valid method for gene expression profiling that has been applied for elucidating key gene pathways associated with ADPKD. In the past 20 years, such studies have revealed many differentially expressed genes (DEGs) and affected signaling pathways in a PKD mouse model [15–20] as well as in ADPKD patients [21, 22]. However, ADPKD is still considered an incurable disease, and knowledge of the pathways involved and its pathogenesis remains incomplete [1, 10, 11]. Most genetic profiling data from ADPKD patients have been uploaded to public databases but not thoroughly analyzed. The data from independent studies are also limited by their source being a single cohort study and by the heterogeneity of samples. Thus, reanalyzing these data with an integrated bioinformatics approach might provide new insights for further research related to novel pharmacological interventions for patients with ADPKD.

One cross-species meta-analysis of conserved biological pathways in ADPKD has been reported [23], and the findings were valuable for further exploration of the potential of combination therapy in ADPKD. However, only a biological analysis was performed, and validation of corresponding genes was not carried out.

In the present study, we accessed 2 original human microarray datasets, GSE7869 [21] and GSE35831 [22], from the NCBI-Gene Expression Omnibus database (NCBI-GEO). Following a previously reported procedure for bioinformatics analysis [24, 25], we extracted DEGs in ADPKD from GSE7869 and GSE35831, separately, and then from among DEGs identified in both datasets, we obtained central node genes from the protein-protein interaction (PPI) network. We then validated the differential expression of identified genes by quantitative

polymerase chain reaction (qPCR) in a *Pkd1* mouse model and ADPKD patient samples. Further analysis of the datasets provided insight into central node genes and enriched pathways in human ADPKD cases. These findings can improve our understanding of the molecular mechanisms of ADPKD with the potential for application in future biological research. Furthermore, these key genes and pathways might be employed as therapeutic targets for ADPKD.

Materials and Methods

Identification of Microarray Data and DEGs

The NCBI-GEO datasets were searched using the term “PKD,” and 6 PKD mouse model datasets, 7 PKD rat datasets, and 2 ADPKD human datasets were retrieved. The 2 original human microarray datasets, GSE7869 and GSE35831, were downloaded. The microarray data of GSE7869 were based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array), and the kidney samples included 5 polycystic kidney specimens resected for medical reasons and noncancerous renal tissue from 3 nephrectomized kidneys with isolated renal cell carcinoma was used as normal control tissue in Canada [21]. The microarray data of GSE35831 [22] were based on the GPL6244 platform (Human Gene 1.0 ST Array), and the human renal tissue samples were acquired from one normal healthy individuals and 2 ADPKD patients in Korea.

The original high-throughput functional genomic expression data were obtained from PubMed as described by Shatakshie Chatterjee work group [23], which was processed in the TXT format. DEGs were identified using *t* tests, and the cutoff criteria were defined as $p < 0.05$ and $|\log FC| > 1$ [26]. Consistently expressed DEGs in the 2 datasets were acquired by Funrich_V3 [27, 28].

Gene Ontology and Pathway Enrichment Analysis

The candidate DEGs were analyzed in terms of the functions and pathways showing enrichment using DAVID 6.7 [26]. Analysis of gene ontology (GO) and KEGG pathway was carried out [29, 30].

Analysis of PPI Network and Modules and Identification of Key Genes and Pathways

Proteins encoded by DEGs were input into STRING online, and a PPI network was obtained in TSV format [25]. Cytoscape software [31] and relative plugins [32] were utilized to process the PPI network. Node degree was calculated with Cytohubba plugins, and hub genes were filtered according to their numbers in the PPI network. Proteins in central nodes were considered as core proteins and key candidate genes that have important physiological regulatory functions.

Validation of Bioinformatics Analysis Result by Quantitative Real-Time PCR

Fluorescence qPCR was carried out to confirm the significantly altered expression of DEGs to verify the reliability and veracity of the bioinformatics analysis results.

Human Kidney Tissue Sample Collection

Renal cortex tissues surrounding a cyst were obtained from ADPKD patients undergoing nephrectomy. As a control group, noncancerous renal cortex tissues were acquired from renal cell carcinoma patients undergoing surgical treatment; malignant cell infiltration was excluded by histology [22]. All tissues were devoid of bacterial, viral, or other contamination and stored in liquid nitrogen before further processing. All human kidney tissues were obtained in accordance with ethical standards and with approval of the Chang-zheng Hospital Ethics Committee (Shanghai, China) in accordance with Helsinki Declaration of 2000. Informed consent was obtained from all patients.

Mouse Kidney Tissue Sample Collection

To generate early-onset ADPKD mouse model, *Pkd1*^{f/f}: tamoxifen-Cre positive mice were given an intraperitoneal injection of tamoxifen (10 mg/kg body weight, formulated in corn oil, T5648, Sigma-Aldrich, St. Louis, MO, USA) on postnatal 10 day (P10) to induce *Pkd1* deletion in the PKD group. *Pkd1*^{f/f}: tamoxifen-Cre negative mice were used as the control group. At 4 weeks of age (P28), mice were anesthetized by ether before death cervical dislocation for collection of kidney samples [17]. To generate late-onset ADPKD mouse model, *Pkd1*^{f/f}: tamoxifen-Cre positive mice were given an intraperitoneal injection of tamoxifen (125 mg/kg body weight, formulated in corn oil) on 2 sequential days (P28 and P29) to induce *Pkd1* deletion in the PKD group. On postnatal 90 and 180 days of age (P90 and P180), mice were anesthetized by ether before death cervical dislocation

Table 1. Primer sets for quantitative real-time PCR

Gene term	Species	Primer F 5'-3'	Primer R 5'-3'
DCN	Mouse	ATGTGGGTGTCAGCTGGATGCG	TGCAGCCCAGGCAAAAGGGT
	Human	AGCTTTGAGGGCTCTGTGGCA	TTGACAGCGGAAGGGGCACA
	Zebrafish	GCTTTTGCTGATCTGAAGAGGGTCT	CTGCGGTCACTTTGGTGATCTTGTT
TAGLN	Mouse	TCAGTGCCTAGGCGGCCTTT	TCGGCTCATGCCGTAGGATGGA
	Human	ATGCCCCGGATGACTTGGCT	TTGGACTGCACTTCGCGGCT
AGXT2	Mouse	ACAGCCGTGTGCTGGCGATT	TCGGGTGGCAGTGACCAACA
	Human	TGGCTCTTTGATGCTGAAGG	AGAAGACGGTGCTTGTATGC
GAPDH	Mouse	AGAACATCATCCCTGCATCC	ATACCAGGAAATGAGCTTGAC
	Human	TAGCCGCATCTTCTTTTGCG	GCGCCAATACGACCAAATC
EHHADH	Mouse	AGAGGTCATTCTAGCCGAT	ACATCCTCTGGCTTACTACCTT
	Human	GCTTCTTCCACTGATCGTCCTC	CCCCACAAATCCAAAACAGTTGC
HADH	Mouse	ACCAGACAAGACCGATTTGCT	ACCAAGAGTCGGTTCACGAT
	Human	TGGCCTCCATTTCTTCAACCC	TCGTTTCATACAGCCTGATTGCTT
ALB β -actin	Mouse	CTGCGCTGAAGCCAATCCTC	TTTCTTGCAGCCTCCACGAGA
	Human	CCAGAATGCGCTATTAGTTCGT	ACTGGCGTTTTTCTCATGCAAC
	Zebrafish	TTACCACCTTACGCGGACTC	GTCACCTTACCGTTCCAGT

ALB, albumin; HADH, hydroxyacyl-CoA dehydrogenase; EHHADH, enoyl-coenzyme A hydratase/3-hydroxyacyl coenzyme A dehydrogenase; AGXT2, alanine-glyoxylate aminotransferase 2; TAGLN, transgelin; DCN, decorin; PCR, polymerase chain reaction.

for collection of kidney samples individually. All kidneys were processed for RNA or protein extraction (flash frozen in liquid nitrogen and stored at -80°C) and histologic examination (fixed in 4% paraformaldehyde buffered solution, pH 7.3–7.4). All mice were raised in specific pathogen-free animal facilities according to a protocol approved by the Animal Care Committee of the Second Military Medical University, Shanghai, China.

Zebrafish Sample Collection

Wild-type zebrafish (Nüsslein-Volhard Laboratory, Tübingen, Germany) were maintained as previously described [33, 34]. Embryos were obtained from natural mating and were kept in E3 solution at 28.5°C [35]. The *pkd2* morpholino (MO) were injected into embryos at the one cell stage to generate ADPKD zebrafish model. Antisense MO oligos were synthesized by Gene Tools (Philomath, OR, USA). Sequences of the *pkd2* MO were used as described: 5'-AGGACGAACGCGACTGGAGCTCATC-3'; and negative control MO was used as described: 5'-CCTCTTACCTCAGTTACAATTTATA-3' [35].

Quantitative Real-Time PCR

Total RNA was extracted from human or mouse kidney tissue samples and zebrafish using Trizol reagent (Takara, Shiga, Japan), according to the manufacturer's instructions. The cDNA was synthesized with 2 μg of total RNA, cDNA synthesis mix (Biomake), and oligo (dT) primers. The primer sets used for the albumin (ALB), enoyl-coenzyme A hydratase/3-hydroxyacyl coenzyme A dehydrogenase (EHHADH), hydroxyacyl-CoA dehydrogenase (HADH), decorin (DCN), transgelin (TAGLN), and alanine-glyoxylate aminotransferase 2 (AGXT2) genes are listed in Table 1. *GAPDH* or β -*ACTIN* was used as the endogenous control. Gene expression data were obtained using a real-time PCR assay (Biomake) and a 7900HT real-time PCR system (Applied Biosystems, Foster City, CA, USA). The relative amount of mRNA compared to the internal control was calculated using the $2^{-\Delta\text{CT}}$ method, in which $\Delta\text{-CT} = -\Delta\text{CT}_{\text{experimental}} - \Delta\text{-CT}_{\text{control}}$ [36].

Fig. 1. Total of 561 consistently expressed genes identified from GSE7869 and GSE35831, including 291 upregulated genes (**A**) and 270 downregulated genes (**B**) in ADPKD kidney tissues compared to control. Different color areas represented different data-sets. The cross areas meant the consistently expressed genes.

Immunohistochemistry Staining

All kidney tissues were embedded in paraffin and cut into a thickness of 3 μ m sections. The kidney sections were deparaffinized and rehydrated. Immunohistochemistry (IHC) staining was performed as described previously [37], with the anti-goat DCN (mouse-AF1060, 1:20, human-AF143, 1:15, R&D, USA).

Statistical Analysis

All data are shown as mean $\pm$ SEM. For data that were normally distributed, comparisons between 2 groups were conducted using the two-tailed *t* test. Wilcoxon tests were used for skewed continuous data. A *p* value <0.05 was regarded as statistically significant. All analyses were conducted using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Graphs were constructed using GraphPad Prism software version 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

Table 2. Total of 561 consistently expressed DEGs identified from GSE7869 and GSE35831, according to integrated bioinformatics analysis, including 291 upregulated genes and 270 downregulated genes in ADPKD kidney tissues, compared to normal kidney tissues

DEGs	Gene name
Upregulated	SAMSN1, OLFML3, CXCL2, FAM129B, SRGN, ATP8B2, NXPE3, BCAT1, ATAD2, LY96, ALDH1L2, RAB31, NDN, EHD2, TTC39C, TNFAIP8, FGF2, EFEMP1, GUCY1A3, CD86, ENO2, RNF144B, RGS1, FOS, FAM43A, SBSPON, LAPTM5, PTCH1, TGFB1, LMO3, PIK3IP1, ST8SIA4, TIMP1, ABCG1, ATRNL1, RPS9, PLD3, APOBEC3C, GIMAP2, FLNA, TAGLN, FGF7, MEF2C, SPON1, CLEC2B, RFTN1, TUSC3, HEPH, EGR1, HDGFRP3, FEZ1, ITGBL1, ANXA1, MDFIC, LHFP, RUNX1T1, BCL6, PDGFD, SPARC, CTSS, DCN, PTPRC, TIMP2, NCF2, KIAA1551, MYH11, DDIT4, DPYSL3, KDELR3, FCGR2B, FXYD1, C1S, ARL4C, GNG11, ACTG2, ECM2, GRK5, MLLT3, CARD6, DOCK11, ADH1C, PDLIM1, CYR61, NR2F2, TMEM43, TPM1, CCDC80, TUBA1A, LRRC17, AMOTL2, ITPRIPL2, LUM, NAP1L1, ADH1B, ID4, NUPR1, DPYSL2, SLPI, VWF, ENTPD1, PDE5A, FMOD, NCKAP1L, CYBRD1, SSPN, KIAA1462, PDGFRL, EVI2B, PKN1, BTG1, RASSF2, PTRF, BMP4, ARRB1, NALCN, FCGR2A, AXL, CD44, MYL9, GSTM5, MFGE8, IGF1, SNX33, PERP, FSTL1, SMOC2, MYO1D, KLF4, SLC4A11, FN1, CCL28, CFH, CXCR4, ELN, C1QA, VIM, TGFB3, SYT11, ADAMTS5, PLK2, CYP1B1, KANK2, MOXD1
Downregulated	PLG, TRPM3, SERPINF2, CTXN3, ZYG11A, PTPN20, CDHR2, CDHR5, HNF4A, PVALB, TFEC, SLC5A11, TUBAL3, PROC, SLC25A42, TCL6, SLC34A3, KCNAB2, SLC7A13, CRIPAK, ZDHHC23, GJB2, SH3YL1, DNASE1, FCAMR, PIPOX, SLC22A8, NAT8B, APOE, HDHD3, AFM, KHK, GLYCTK, UPP2, LEAP2, MSRA, SLC39A5, PCK2, SLC5A2, ECHS1, CTSA, SNX30, ANKS4B, SLC22A12, CYP2B6, PRODH2, AGMAT, PDZK1IP1, CCDC88C, CLNK, HAO2, NQO2, AGT, ACSF2, SLC22A5, HNF4G, DMRT2, A1CF, ABCC2, CYP8B1, FARP1, ECHDC2, SLC23A1, SLC13A3, ANK2, MT1F, SLC2A9, ANGPTL3, TRPM6, AHCY, MIOX, HAVCR2, GCH1, SLC12A6, ALDOB, LGMN, SLC22A11, ACO1, MAPT, TTC38, CYP4F2, TMEM174, TMED6, CDH9, SLC9A3R1, ACOX2, DIO1, TMEM207, IGSF11, SLC16A1, SLC25A5, PPARA, SLC2A2, GLB1L, SLC7A9, AKR1A1, TPCN2, FTCD, HIBCH, MYOM3, CYP4F3, VIL1, PDSS1, CYP27B1, SLC22A6, SLC13A2, SLC5A12, SYT13, STRIP2, CYP17A1, SLC6A19, SLC10A2, SLC22A7, AGXT2, SLC5A9, SLC28A1, PLA2G12B, SLC23A3, SLC16A10, SLC22A13, SLC1A1, SLC7A7
DEGs, differentially expressed genes; ADPKD autosomal dominant polycystic kidney disease.	

Results

Identification of DEGs in ADPKD

In the comparison of gene expression between ADPKD tissues and normal kidney tissues, 6,422 and 1,152 DEGs extracted from the expression profile datasets GSE7869 and GSE35831, respectively. By utilized Funrich_V3, 561 DEGs were identified in ADPKD kidney tissues, which were consistently expressed in both GSE7869 and GSE35831, including 291 upregulated genes and 270 downregulated genes, compared to normal kidney tissues (Fig. 1; Table 2).

GO Analysis of DEGs

The functions and pathways of candidate DEGs were obtained by using multiple online websites, including DAVID, KEGG pathway, and GO, and the cutoff criterion was $p < 0.05$ [26, 29, 30]. GO analysis was performed with DAVID. The DEGs were classified into 3 functional groups [29, 30]: molecular function, biological process, and cellular component (Fig. 2, 3), and the DEGs that showed significant enrichment are listed in Table 3.

In the biological process group, the upregulated genes (Fig. 2A, 3A) were mainly involved in extracellular matrix (ECM) organization, cell adhesion, ECM disassembly, muscle contraction, and wound healing. In the molecular function group, the upregulated genes were mainly linked to protein binding, heparin binding, collagen binding, and calcium ion binding. In the cellular

Fig. 2. GO analysis of consistently expressed DEGs in ADPKD. Upregulated DEGs (**A**) and downregulated DEGs (**B**) were individually classified into 3 groups: biological process (white), molecular function (gray), and cellular component (black). GO, gene ontology.

Fig. 3. Significant Enriched Go Terms of consistently expressed DEGs in ADPKD based on their functions, including upregulated DEGs (**A**) and downregulated DEGs (**B**). GO, gene ontology.

Table 3. The significant enriched analysis of DEGs in ADPKD

Term	Description	Count	p value
Up-regulated			
GO:0031012	Extracellular matrix	31	1.6658E-16
GO:0005518	Collagen binding	15	2.74844E-13
GO:0030198	Extracellular matrix organization	22	3.69419E-12
GO:0005578	Proteinaceous extracellular matrix	24	2.26852E-11
GO:0005615	Extracellular space	55	3.72548E-11
GO:0022617	Extracellular matrix disassembly	14	1.46593E-10
GO:0070062	Extracellular exosome	84	5.73833E-10
GO:0005576	Extracellular region	58	1.13345E-09
GO:0005925	Focal adhesion	25	7.56565E-09
GO:0008201	Heparin binding	16	2.6642E-08
Down-regulated			
GO:0070062	Extracellular exosome	116	9.39062E-29
GO:0016324	Apical plasma membrane	31	1.99271E-17
GO:0031526	Brush border membrane	13	5.59467E-12
GO:0055085	Transmembrane transport	20	2.83982E-09
GO:0005903	Brush border	11	1.22805E-08
GO:0006814	Sodium ion transport	11	2.55632E-07
GO:0005782	Peroxisomal matrix	9	2.59143E-07
GO:0005887	Integral component of plasma membrane	45	8.17944E-07
GO:0008152	METABOLIC process	14	1.03062E-06
GO:0005759	Mitochondrial matrix	19	1.23057E-06

DEGs, differentially expressed genes; ADPKD autosomal dominant polycystic kidney disease.

Fig. 4. Significant enriched pathway terms of DEGs (upregulated, white; downregulated, black).

component group, the upregulated genes were mainly related to the extracellular exosome, extracellular region, proteinaceous ECM, extracellular space, and focal adhesion.

In the biological process group, the downregulated genes (Fig. 2B, 3B) were mainly involved in transmembrane transport, oxidation–reduction process, metabolic process, sodium ion transport, and fatty acid β -oxidation. In the molecular function group, the downregulated genes were mainly associated with protein homodimerization activity, identical protein binding, catalytic activity, transporter activity, oxidoreductase activity, hydrolase activity, and receptor binding. In the cellular component group, the downregulated genes were mainly related to the extracellular exosome, integral component of the membrane, plasma membrane, cytosol, integral component of the plasma membrane, mitochondrion, apical plasma membrane, and endoplasmic reticulum membrane.

Signaling Pathway Enrichment Analysis

The functional and signaling pathways enriched in association with the DEGs were investigated through the KEGG pathway and GO online tools (Fig. 4; Table 4). The upregulated DEGs were mainly related to *Staphylococcus aureus* infection, pertussis, phagosome, pathways in cancer, PI3K-Akt signaling pathway, focal adhesion, proteoglycans in cancer, complement, and coagulation cascades. The downregulated DEGs were mainly involved in metabolic pathways; biosynthesis of antibiotics; carbon metabolism; fatty acid degradation; β -alanine metabolism; lysine, valine, and (iso) leucine degradation; glycine, serine, and threonine metabolism; and biosynthesis of amino acids.

PPI Network and Modular Analysis of Key GENES and Pathways

The String online database [25] and Cytoscape software [38] were used for PPI and modular analysis. A total of 561 consistently expressed DEGs (291 upregulated and 270 downregulated genes) were individually filtered into the PPI network, and 421 nodes and 1,776 edges (Fig. 5A) were obtained. From the 421 nodes, 34 central node genes were acquired, and the 10 most significant node genes were *ALB*, *JUN*, *insulin-like growth factor 1*, *fibroblast growth factor 2*, *alpha smooth muscle actin 2*, *plasminogen*, *EHHADH*, *vimentin*, and *DCN*. According to the degree of importance, 2 significant modules were selected from the PPI network for further analysis using Cytotype MCODE. Pathway enrichment analysis showed that 23 nodes and 140 edges (Fig. 5B;

Table 4. Signaling pathway enrichment analysis of DEGs function in ADPKD

KEGG pathway	Name	Gene count	p value	Genes
<i>Upregulated DEGs</i>				
hsa05205	Proteoglycans in cancer	11	6.21E-03	CAV1, CD44, LUM, HSPG2, IGF1, PTCH1, DCN, FGF2, FLNA, HOXD10, FN1
hsa05202	Transcriptional misregulation in cancer	10	6.00E-03	MEF2C, CD86, CDKN1B, NUPR1, CCND2, RUNX1T1, IGF1, BCL6, IGFBP3, MLLT3
hsa05200	Pathways in cancer	17	4.62E-03	BMP4, FGF7, RUNX1T1, TGFB3, IGF1, GNG11, BIRC3, FOS, CDKN1B, LAMA3, CXCR4, JUN, PDGFRA, GNB4, PTCH1, FGF2, FN1
hsa04151	PI3K-Akt-signaling pathway	16	3.31E-03	FGF7, PKN1, IGF1, GNG11, DDIT4, ITGA9, VWF, CDKN1B, LAMA3, CCND2, PDGFRA, COL6A2, GNB4, PDGFD, FGF2, FN1
hsa04145	Phagosome	10	3.25E-03	MSR1, FCGR2B, NCF2, C3, NCF4, ITGB2, C1R, FCGR2A, CTSS, TUBA1A
hsa05140	Leishmaniasis	8	4.77E-04	FOS, NCF2, C3, JUN, NCF4, TGFB3, ITGB2, FCGR2A
hsa04610	Complement and coagulation cascades	8	4.00E-04	C1QA, VWF, C3, CFH, SERPING1, C1R, C1S, C1QC
hsa04510	Focal adhesion	14	2.07E-04	CAV1, IGF1, BIRC3, FLNA, MYL9, VWF, ITGA9, LAMA3, CCND2, JUN, PDGFRA, COL6A2, PDGFD, FN1
hsa05133	Pertussis	10	1.51E-05	C1QA, FOS, C3, LY96, JUN, SERPING1, ITGB2, C1R, C1S, C1QC
hsa05150	<i>Staphylococcus aureus</i> infection	9	9.39E-06	C1QA, FCGR2B, C3, CFH, ITGB2, C1R, FCGR2A, C1S, C1QC
<i>Downregulated DEGs</i>				
hsa00380	ACS metabolism	6	8.17E-04	GCDH, ALDH1B1, EHHADH, ECHS1, KMO, HADH
hsa00260	Glycine, serine, and threonine metabolism	6	7.26E-04	CHDH, AGXT2, DAO, GRHPR, PSAT1, PIPOX
hsa00310	Lysine degradation	7	3.79E-04	GCDH, ALDH1B1, EHHADH, ECHS1, AASS, HADH, PIPOX
hsa01230	Biosynthesis of amino acids	9	6.36E-05	ASS1, MAT2A, ACO1, ALDOB, PKLR, IDH2, PSAT1, GPT2, PC
hsa00280	Valine, leucine, and isoleucine degradation	8	2.27E-05	ALDH1B1, EHHADH, ECHS1, ABAT, HIBCH, HADH, PCCB, ACAA1
hsa00410	Beta-alanine metabolism	7	1.89E-05	ALDH1B1, EHHADH, UPB1, ECHS1, ABAT, DPYS, HIBCH
hsa00071	Fatty acid degradation	8	1.45E-05	GCDH, CYP4A11, ACOX1, ALDH1B1, EHHADH, ECHS1, HADH, ACAA1
hsa01200	Carbon metabolism	13	1.07E-06	ACO1, EHHADH, HAO2, ALDOB, PKLR, IDH2, ECHS1, RGN, HIBCH, PSAT1, PCCB, GPT2, PC
hsa01130	Biosynthesis of antibiotics	19	6.14E-08	GCDH, ASS1, ACO1, EHHADH, ALDOB, ECHS1, AASS, PCK2, AKR1A1, ALDH1B1, HAO2, PKLR, IDH2, RGN, DAO, PSAT1, HADH, PCCB, ACAA1
hsa01100	Metabolic pathways	65	5.92E-17	ACOX2, ACOX1, GDA, EHHADH, SAT2, ANPEP, RGN, DAO, HADH, AGPAT3, GPT2, HPD, ACO1, ACSM2A, QDPR, GRHPR, PNPLA3, G6PC, ALDH1B1, PKLR, HAO2, ABAT, PCCB, ACAA1, ALPL, AHCY, ASS1, CYP2B6, FUT6, ALDOB, DPYS, ECHS1, AASS, UPP2, KMO, ATP6V1B2, AGMAT, PIPOX, GCH1, CYP27B1, AKR1A1, PLA2G12B, IDH2, PNPO, ETNK2, UGT2A3, GCDH, CHDH, MAT2A, KL, UPB1, FTCD, PCK2, KHK, GBA2, CYP4A11, CYP17A1, PRODH2, AGXT2, CYP4F3, HIBCH, CYP4F2, PSAT1, CYP8B1, PC

DEGs, differentially expressed genes; ADPKD autosomal dominant polycystic kidney disease.

Fig. 5. PPI network complex and modular analysis of different express genes. **A** Total of 561 DEGs (291 up-regulated genes in red and 270 down-regulated genes in blue) filtered into the DEGs PPI network complex by using the STRING online database, includes of 421 nodes and 1,776 edges. **B** Module 1 includes of 23 nodes and 140 edges. **C** Module 2 includes of 11 nodes and 39 edges.

Table 5. Pathway enrichment analysis of module 1 gene function

Term	Description	Count	p value
GO:0005515	Protein binding	19	0.0000759
GO:0005576	Extracellular region	16	1.13E-14
GO:0005615	Extracellular space	14	9.92E-13
GO:0002576	Platelet degranulation	13	7.52E-27
GO:0031093	Platelet alpha granule lumen	12	3.02E-28
GO:0070062	Extracellular exosome	10	0.000178225
GO:0005886	Plasma membrane	8	0.038480439
GO:0042802	Identical protein binding	7	0.000000342
GO:0007568	Aging	6	8.75E-10
GO:0042060	Wound healing	5	4.50E-10
GO:0005777	Peroxisome	5	2.41E-09
GO:0072562	Blood microparticle	5	1.39E-08
GO:0008083	Growth factor activity	5	3.22E-08
GO:0030198	Extracellular matrix organization	5	8.95E-08
GO:0008284	Positive regulation of cell proliferation	5	0.0000135
GO:0043231	Intracellular membrane-bounded organelle	5	0.0000365
GO:0009986	Cell surface	5	0.0000393
GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	5	0.000788458
GO:0005739	Mitochondrion	5	0.003013631
hsa05200	Pathways in cancer	5	0.000322875
hsa00071	Fatty acid degradation	4	6.11E-08
hsa03320	PPAR signaling pathway	4	0.000000608
hsa04610	Complement and coagulation cascades	4	0.0000012
hsa04146	Peroxisome	4	0.00000154
hsa04510	Focal adhesion	4	0.000117309
hsa04151	PI3K-Akt signaling pathway	4	0.001318973

GO, gene ontology.

Table 6. Pathway enrichment analysis of module 2 gene function

Term	Description	Count	p value
GO:0005739	Mitochondrion	8	1.06E-09
hsa00280	Valine, leucine, and isoleucine degradation	5	2.02E-12
hsa00071	Fatty acid degradation	4	4.18E-10
GO:0005524	ATP binding	4	0.00092
hsa00380	Tryptophan metabolism	3	6.17E-08
hsa00640	Propanoate metabolism	3	2.44E-08
hsa00310	Lysine degradation	3	1.82E-07
hsa01212	Fatty acid metabolism	3	1.31E-07
hsa01200	Carbon metabolism	3	4.20E-06
hsa00650	Butanoate metabolism	3	1.61E-08
hsa00410	Beta-alanine metabolism	3	2.13E-08
GO:0006631	Fatty acid metabolic process	3	1.88E-08

GO, gene ontology.

Fig. 6. DCN expression in ADPKD renal tissues. **A** DCN mRNA expression values (sample values for ID = 209335_at) in GSE7869. DCN mRNA expression values were upregulated in small, medium and large cysts groups compared to control and minimally groups. Data are expressed as mean $\pm$ SEM; ** $p < 0.01$ versus or Control or minimally groups. **B** DCN mRNA expression in ADPKD zebrafish model. DCN mRNA expressions were downregulated in zebrafish model of ADPKD (pkd2 morphants) groups compared to control groups after 24 hpf (hour post fertilization) and 72 hpf. Data are expressed as mean $\pm$ SEM; * $p < 0.05$ versus Control 24 hpf; # $p < 0.05$ versus control 72 hpf. **C** Renal DCN mRNA expressions in late-onset ADPKD mouse model. On P90, renal DCN mRNA expressions were downregulated in Pkd1 mouse group compared to WT group. On P180, renal DCN mRNA expressions were upregulated in Pkd1 mouse group compared to WT group. Data are expressed as mean $\pm$ SEM; * $p < 0.05$ versus WT; # $p < 0.05$ versus WT. **D–K** Renal DCN protein expression in ADPKD. In ADPKD early-onset mouse model, a marked increased expression of DCN protein was observed in renal interstitial tissue compared to WT mouse on postnatal 28 day (P28; **D, E**). In ADPKD patient renal tissue, significant increased expression of DCN protein was observed in renal interstitial tissue and CLECs compared to control renal tissue (**F, G**). There was no difference on DCN protein expression in renal tissue between ADPKD late-onset mouse and WT mouse on P90 (**H, I**). In ADPKD late-onset mouse model, increased expression of DCN protein was observed in CLECs and renal interstitial tissue compared to WT mouse on P180 (**J, K**; $\times 400$). DCN, decorin.

Table 5) constituted module 1 and were associated with protein binding, extracellular region, extracellular space, platelet degranulation, extracellular exosome, plasma membrane, and aging. Module 2 consisted of 11 nodes and 39 edges associated with mitochondrion, degradation of valine and (iso) leucine, fatty acid degradation, ATP binding, tryptophan metabolism, propanoate metabolism, lysine degradation, fatty acid, and carbon metabolism (Fig. 5C; Table 6).

Fig. 7. Quantitative real-time PCR of renal tissue in Pkd1 mouse model and ADPKD human compared to WT and control. Data are expressed as mean $\pm$ SEM; * $p < 0.05$ vs. WT or Control. DCN, decorin; TAGLN, transgelin; ALB, albumin; AGXT2, alanine-glyoxylate aminotransferase 2; EHHADH, enoyl-coenzyme A hydratase/3-hydroxyacyl coenzyme A dehydrogenase; HADH, hydroxyacyl-CoA dehydrogenase.

DCN mRNA expression values (sample values for ID = 209335_at) were reanalyzed in GSE7869 according to different developmental stage of cysts formation, including control, minimally cystic tissue, small, medium, and large cysts [21]. DCN mRNA expression values were upregulated in small, medium, and large cysts groups compared to control and minimally groups. No difference was observed between control and minimally cystic tissue (Fig. 6A).

Validation of Selected DEGs

Quantitative Real-Time PCR

Using qPCR in kidney samples affected by PKD and normal kidney samples from a mouse model (early-onset ADPKD) and humans, we confirmed the differential expression of DEGs identified via our bioinformatics analysis, including: *ALB*, *EHHADH*, *HADH*, *DCN*, *TAGLN*, and *AGXT2* (Fig. 7). The expression levels of *DCN* and *TAGLN* were significantly higher in PKD kidney samples from both humans and mice compared to levels in control samples. The expression levels of *ALB*, *EHHADH*, *HADH*, and *AGXT2* were significantly lower in PKD kidney samples from both humans and mice compared to levels in control samples.

ADPKD zebrafish model was used to detect *DCN* mRNA expression in different stages of cyst formation. *DCN* mRNA expressions were upregulated more than 2 folds in control groups compared to zebrafish model of ADPKD (*pkd2* morphants) groups at 24 and 72 hpf (hour post fertilization; Fig. 6B).

Kidney *DCN* mRNA expressions were detected in late-onset ADPKD mouse model. *DCN* mRNA of mice kidneys was quantified by qPCR on P90 and P180 individually. On P180, *DCN* mRNA expressions were upregulated more than 2 folds in *Pkd1* mouse group compared to wide type group (Fig. 6C). On P90, *DCN* mRNA expressions were downregulated in *Pkd1* mouse group compared to wide-type group, but the change is no more than 2 folds.

IHC Staining

Renal *DCN* expression in ADPKD renal tissues was performed in mouse models and human with IHC. In ADPKD early-onset mouse model, a marked increased expression of *DCN* protein was observed in renal interstitial tissue compared to WT mouse on age of P28 (Fig. 6D, E). In ADPKD late-onset mouse model, increased expression of *DCN* protein was observed in cyst line epithelial cells and renal interstitial tissue compared to WT mouse on age of P180 (Fig. 6J, K). There was no difference on *DCN* protein expression in renal tissue between ADPKD late-onset mouse and WT mouse on P90 (Fig. 6H, I). In ADPKD patient renal tissue, significant increased expression of *DCN* protein was observed in renal interstitial tissue and cyst line epithelial cells compared to control renal tissue (Fig. 6F, G).

Discussion

Our reanalysis of 2 gene profiling datasets from ADPKD and normal human kidney tissues revealed 561 consistent DEGs (291 upregulated genes and 270 downregulated genes) in ADPKD. Most of upregulated DEGs indicated enrichment of pathways involved in cancer, PI3K-Akt signaling pathway, focal adhesion, complement, and coagulation cascades. Most of downregulated DEGs were associated with enrichment of metabolic pathways, including those responsible for carbon, fatty acid, and amino acid metabolism. The generated PPI network revealed 421 nodes (DEGs), and of these, the 10 most significantly DEGs were validated by qPCR analysis in both a *Pkd1* mouse model and ADPKD patient samples.

More specifically, the upregulated DEGs were mainly involved in pathways of ECM organization, ECM disassembly, cell adhesion, collagen and integrin binding, protein binding, ECM, and focal adhesion. These findings are consistent with observations in cystic kidneys. For example, Drummond's [39] review of the characteristics of fibrosis observed in cystic kidney diseases provided evidence of altered interactions between epithelial cells and stromal fibroblasts, followed by accumulation of ECM (collagen, specific laminins, and other proteins) and enhanced expression of matrix metalloproteases and transforming growth factor β . Okada et al. [40] investigated the phenotype of collagen-producing cells in the cystic kidneys of DBA/2-*pcy* mice and characterized the spectrum of interstitial cells associated with renal fibro-

genesis. Furthermore, Lee et al. [41] provided genetic evidence that a functional integrin- β 1 is required for the early events leading to renal cystogenesis in ADPKD and suggested that the integrin signaling pathway may represent an effective therapeutic target for slowing disease progression.

DCN was among the 10 most significantly DEGs and was significantly upregulated in human ADPKD kidney samples. Our experiments validated that *DCN* was upregulated in human ADPKD kidney samples and in late stage (P180) of late-onset ADPKD mouse model. The protein product of *DCN* is a member of the small leucine-rich proteoglycan family of proteins [42]. Diseases associated with *DCN* protein include corneal dystrophy, congenital stromal, and stromal dystrophy [43]. Previous research showed that *DCN* suppresses tumorigenic growth, angiogenesis, and prevents metastatic lesions in a variety of in vitro and in vivo tumor models and thus represents a promising therapeutic candidate for combatting solid malignancies [44]. In the normal kidney, *DCN* presents mainly in the renal interstitium [45]. *DCN* accumulated in tubular interstitial in several nephropathy appeared to play a crucial role in renal inflammation and fibrogenesis, predicting progression of chronic kidney disease [42]. Soluble *DCN* is regarded as a signaling molecule and an endogenous ligand of TLR2/TLR4 in renal inflammation [46, 47] and appears to be a potent antifibrosis molecule, acting against renal fibrogenesis directly through receptor-mediated signaling and indirectly in transforming GF β -dependent and - independent pathways [42]. In unilateral ureteral obstruction mouse, the absence of *DCN* aggravated renal fibrosis. According to our results, we can make a conclusion that *DCN* accumulation in ADPKD renal interstitium is associated with the development of cysts in ADPKD. Whether *DCN* takes part in the fibrosis progression or acts against fibrogenesis in ADPKD is not known, and further experiments are necessary. However, *DCN* was shown decreased in the zebrafish *Pkd2*-MO model and in the early-stage (P90) of late-onset ADPKD mouse model. *DCN* mRNA expressions were downregulated significantly in the zebrafish *Pkd2*-MO model at different stages of cyst formation. We can speculate that the downregulated *DCN* is associated with the formation of cysts in ADPKD and that *DCN* might be a retardant during cysts formation. The cross-breeding between *DCN* knockout mice and *Pkd1* conditional knockout mice would be necessary to confirm the unique role of *DCN* in cysts formation and development in ADPKD.

The downregulated DEGs identified in the present study mostly indicated enrichment of metabolic pathways, such as those for carbon metabolism, fatty acid degradation, and amino acid metabolism. In PPI network, module 2 consisted of 11 nodes, mainly associated with mitochondrion and ATP binding. This finding supports the hypothesis that ADPKD pathogenesis could be causally linked to altered cellular metabolism [12]. A study by Menezes et al. [17] demonstrated that metabolic pathways are key elements in postnatal kidney maturation and early steps of cyst formation in a *Pkd1* mouse model of ADPKD. Moreover, a mutation in the *PKD1* gene was shown to cause enhanced glycolysis in cells, in a murine model of PKD as well as and in human ADPKD kidneys [48]. Another study also revealed defective glucose metabolism in ADPKD and showed that treatment with 2-deoxy-D-glucose could ameliorate the progression of PKD [49]. These findings led to a new therapeutic approach of targeting the glycolytic pathway for treatment of PKD. At the same time, impaired fatty acid oxidation was discovered in an orthologous mouse model of ADPKD [19]. This was supported by a clinical study that obtained evidence of perturbation in fatty acid metabolism early in ADPKD patients, even in those with preserved kidney function [50]. From these results, the authors concluded that the LOX (5- and 12/15-lipoxygenase) pathways may be potential therapeutic targets for slowing the progression of ADPKD.

Some of the downregulated DEGs and corresponding pathways identified in the present study were associated with amino acid metabolism, such as pathways in amino acid (trans-membrane) transport, biosynthesis of amino acids, beta-alanine metabolism, and lysine,

valine, leucine, and isoleucine degradation. Branched-chain amino acids (BCAAs), including leucine, play crucial roles in the activation of the mammalian target of rapamycin pathway [51], and when Yamamoto et al. [52] administered BCAAs, including leucine, dissolved in the drinking water to a *Pkd1* mouse model, they found that BCAAs accelerated ADPKD progression via the mammalian target of rapamycin and MAPK/ERK pathways. Thus, BCAAs may be harmful to patients with ADPKD. Moreover, amino acid metabolism is tightly linked to ADPKD progression. In the present study, we validated the downregulated expression of the *EHHADH* and *HADH* genes in PKD kidney samples compared to control kidney samples. Both of these genes are associated with metabolic pathways, including those for carbon metabolism, fatty acid degradation, and amino acid degradation. Interestingly, mistargeting of peroxisomal EHHADH was found to disrupt mitochondrial metabolism and leads to renal Fanconi's syndrome [53].

Raptis et al. [54] summarized the role of the endothelium in ADPKD. Nitric oxide (NO) bioavailability and NO-dependent vasodilation are affected in patients with ADPKD. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO and a putative biochemical marker of atherosclerosis [55]. ADPKD patients with an enhanced glomerular filtration rate >60 mL/min/1.73 m² had higher serum ADMA levels, as well as higher levels of several other endothelial dysfunction markers, than healthy controls [56]. Several studies have noted a possible harmful role of ADMA in ADPKD [57, 58]. AGXT2 can metabolize ADMA, and Rodionov et al. [59] found that AGXT2 may play a major role in the maintenance of ADMA homeostasis in the setting of local or systemic elevation of ADMA in mice after bilateral nephrectomy. Our results showed that *AGXT2* gene expression was lower by >20 -fold in ADPKD human kidney samples compared with control tissues and lower by 2-fold in PKD mice kidney samples compared with wild-type kidney tissues. Thus, we can hypothesize that the increased serum ADMA level in ADPKD patients may correspond with a decline in *AGXT2* expression in the affected kidneys. Further research is needed to elucidate the mechanism of this phenomenon.

Our bioinformatics results interestingly also showed that *ALB* gene expression was significantly lower in ADPKD kidney samples, and this finding was confirmed by qPCR analysis of mRNA expression. ALB is known to act as a carrier protein for a wide range of endogenous molecules including hormones, fatty acids, and metabolites, as well as exogenous drug [60]. The *ALB* gene has pleiotropic effects of the human serum ALB levels in health and disease, with one study reporting that serum ALB might have a direct role in the inhibition of hepatocellular carcinoma growth either through modulation of α -fetoprotein or through its action on growth controlling kinases [61]. Yoo et al. [62] demonstrated that ALB expression is required for adipocyte differentiation of 3T3-L1 cells. Additionally, the *ALB* gene-encoded preproprotein is proteolytically processed to generate the mature protein, and a peptide derived from this protein, EPI-X4, is an endogenous inhibitor of the CXCR4 chemokine receptor [63]. Our analysis showed that the *ALB* gene was downregulated by more than 10-fold in PKD kidney samples compared to control kidney samples, and the mechanism of this alteration and the function of renal ALB protein are unclear in PKD.

A cross-species meta-analysis provided profiling of conserved biological pathways in ADPKD, using data from 9 ADPKD microarray datasets from rats, mice, and humans [23], and their findings supported the possibility of exploring the potential of combination therapy in ADPKD. In our work, although data were only available from 2 gene profiling datasets on clinical samples from ADPKD patients in the GEO database, which greatly limits the effectiveness and scope of the bioinformatics analysis, we identified and validated the downregulation of genes in metabolic pathways, such as amino acid degradation, and this finding may benefit ADPKD research. Additional limitations in our work also include the sample size in GSE35831, which includes data for only 1 normal and 2 ADPKD human renal tissue samples. Additionally, the use of renal samples from ADPKD patients with ESRD may have an impact

on subsequent analysis, as some changes in gene expression may be attributable to ESRD. Moreover, although mutations in the *Pkd1* or *Pkd2* gene are predominantly responsible for ADPKD, the focal and sporadic nature of individual cystogenesis suggests the involvement of another molecular mechanism such as epigenetic alteration [22] or miRNA-mediated regulation [20]. It seems likely that a combinatory and/or serial approach at different stages of disease will be required to more completely explore the mechanism of ADPKD [12].

Conclusion

The presented bioinformatics analysis of ADPKD gene profiling datasets identified multiple DEGs and enriched signaling pathways in human ADPKD. The abnormal expression of DCN may provide a new therapeutic target for ADPKD. Our results indicate that metabolism regulation and mitochondrion function in ADPKD also may become targets for future research related to therapeutic interventions. Further biological validation of the expression patterns, functions, and mechanisms of the identified DEGs is warranted.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2016YFC0901502), National Natural Science Foundation of China (81873595, 81670612), Shanghai Top Priority Key Clinical Disciplines Construction Project (2017ZZ02009).

Disclosure Statement

The authors declare that there are no conflicts of interest.

The results presented in this paper have not been published previously, in whole or part, except in abstract format.

Author Contributions

C.M. conceived and coordinated the study. D.L. wrote the paper. C.X. revised the paper. Y.H., D.L., and B.Y. carried out the bioinformatics analysis. L.B., S.S., and L.F. collected the human renal samples. D.L. performed the animal experiment and carried out the real-time PCR, IHC and performed the statistics analysis. S.C. and D.X. performed the zebrafish experiment. All authors reviewed the results and approved the final version of the manuscript.

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